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Unusual arrangement and behaviour of the sex chromosomes of *Aphodius* (*Agolius*) *abdominalis* Bonelli, 1812, and comparison with *A.* (*A.*) *bonvouloiri* Harold, 1860 (Coleoptera: Aphodiidae)

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Abstract. *Aphodius abdominalis* Bonelli, 1812 is shown to have a karyotype comprising nine pairs of autosomes and sex chromosomes which are X0 (male), XX (female). At first metaphase of meiosis the X chromosome is linked to an autosomal bivalent by a darkly staining area of the cytoplasm, resembling the Xy_p arrangement typical of *Aphodius* species, but giving nine, rather than 10, elements in the nucleus. C-banding, which shows the centromeres, confirms this unusual arrangement. *A. bonvouloiri*, the only other known species of subgenus *Agolius* Mulsant et Rey, 1869, has a male karyotype with nine pairs of autosomes and Xy sex chromosomes. No preparations of its meiosis are available.

Key words: *Aphodius*, *Agolius*, karyotypes, sex chromosomes, Xy_p, X0, C-banding, X-autosomal trivalent.

Introduction

The genus *Aphodius* Illiger, 1798, in the broad sense (i.e. not accepting the elevation of the subgenera to generic rank as proposed by Dellacasa et al., 2000) comprises more than 1600 species world-wide (estimate based on Dellacasa, 1987). Approximately 65 of these species are known chromosomally (Smith, Virkki, 1978; Wilson, 2001, 2002; Wilson, Angus, 2003, 2004 a, b, 2005, 2006 a, b; Maté, Angus, 2005, and some unpublished data) and although they show interesting and taxonomically useful differences between species, the general karyotypes are very uniform with 2n = 18 + Xy (♂), with the sex chromosomes showing the common Polyphagan "parachute" association (Xy_p) during first division of meiosis. There is some minor variation in chromosome number. Thus Yadav (1973) recorded 2n = 22 (20 + Xy_p) for *A. moestus* (Fabricius, 1801) but Yadav et al. (1993) recorded the usual number of 20 (including the sex chromosomes) for this species. Wilson and Angus (2004b) recorded up to six B-chromosomes in *A. foetidus* (Herbst, 1783) in addition to the normal diploid karyotype of 18 + Xy_p, and suggested that the presence of B-chromosomes was the most likely explanation for the variation in chromosome number recorded for *A. moestus*. Maté and Angus (2005) recorded up to three B-chromosomes in *A. niger* Illiger, 1798 and one B-chromosome in *A. wilsonae* Maté et Angus, 2005. Variation in the sex chromosomes involves a wide range of size

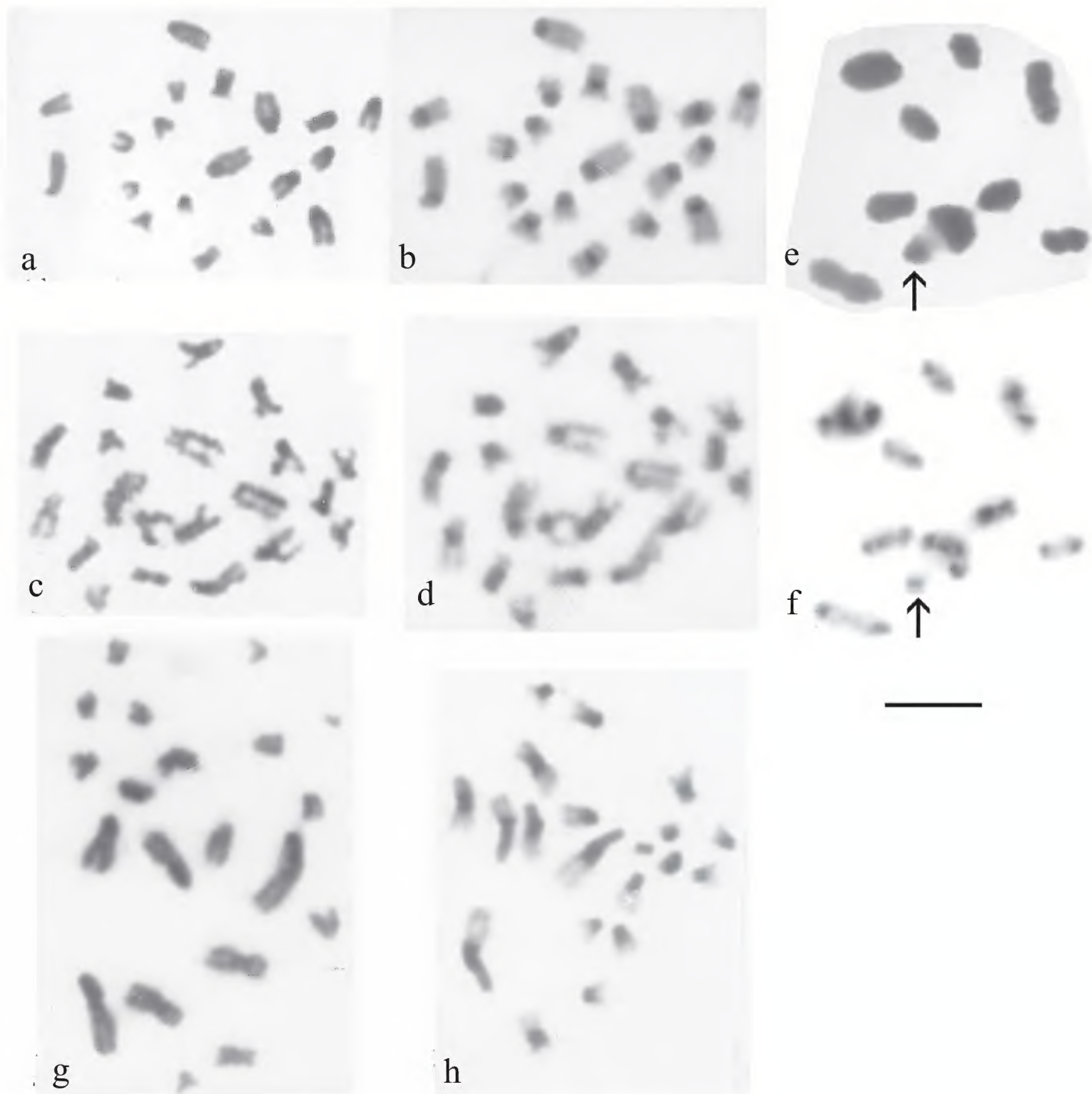

Fig. 1, a-h. Mitotic (**a-d, g, h,**) and meiotic (**e, f**) chromosomes of *Aphodius* spp. **a** - *A. abdominalis*, ♂, mid-gut cell, plain. **b** - the same nucleus, C-banded. **c** - *A. abdominalis*, ♀, mid-gut cell, plain. **d** -the same nucleus, C-banded. **e** - *A. abdominalis*, ♂, meiosis, metaphase I, plain. **f** - the same nucleus C-banded. The X chromosome is arrowed. **g** - *A. bonvouloiri*, ♂, mid-gut, plain. **h** - *A. bonvouloiri*, ♂, mid-gut, C-banded. Bar = 5 µm.

of the X chromosome, but much less in the y chromosome, which is generally small to dot-like. However, Maté and Angus (2005) found that in *A. niger* the y chromosome was as large as the middle-range autosomes, but that it still showed the parachute association with the X at first metaphase of meiosis. Wilson and Angus (2006a) failed to find a y chromosome in *A. nemoralis* Erichson, 1848 but considered this to be an artefact resulting from limited material. It is against this background that the surprising results of this study are presented:

male *A. abdominalis* not only lacks a y chromosome, but the X chromosome associates with an autosomal bivalent during first division of meiosis.

Material and Methods

The *Aphodius abdominalis* were collected in Switzerland, Canton of Valais, Alps above St Luc and Chandolin, floating in lakes and pools at altitudes of 2300-2600 metres, 19-22 August 2008 and 21-23 July 2009. Some attempt was made to find material in cow dung, but without success. The analysed material comprises 4 ♂♂ and 1 ♀. The *A. bonvouloiri* were collected in Spain, Provincia de Madrid, southern slope of Mt Peñalara by the Puerto del Paular o de los Cotos, cow and horse dung at altitude of 1800 metres, 2 June 2007. The analysed material comprises 1 ♂ and 1 ♀.

The methods of chromosome preparation and staining were as outlined by Dutton and Angus (Dutton, Angus, 2007, Angus, 2008). The beetles are in R. B. Angus' collection.

Results

A. abdominalis Bonelli, 1812

Published information: none. 2n = 18 + X0 (♂), XX (♀). Male mitotic chromosomes, from mid-gut, are shown in Fig. 1, a (plain) and b (the same nucleus, C-banded), and female mitotic chromosomes, plain and C-banded, in Fig. 1, c, d. Karyotypes from these nuclei are shown in Fig. 2, a, b and d, e, while Fig. 2, c shows a plain karyotype (♂) from a different nucleus, with the chromosomes less condensed. The karyotype comprises three pairs of fairly long acrocentric autosomes, then a medium-sized pair of metacentrics, followed by four more pairs of acrocentric or subacrocentric autosomes of decreasing size. The X chromosome is subacrocentric, about the same size as autosome 9. There is no y chromosome. C-banding shows distinct centromeric C-bands, and there are indications of secondary constrictions (NORs) on the short arms of autosomes 4 and 5 of the female karyotypes (Fig. 2, d, e), but these are not apparent in the males illustrated. First metaphase of meiosis is shown in Fig. 1, e (plain) and Fig. 1, f (C-banded). The X chromosome is indicated by an arrow (↑). The nucleus contains nine elements, one of which, the sex chromosome element, looks like Xy_p in the plain nucleus, with a darkly staining cytoplasmic region between the larger and smaller chromosomes. The true nature of this arrangement is revealed by C-banding. The pairs of centromeres of the autosomal bivalents are very clear, and a similar pair of centromeres is also present in the larger part of the sexual element, showing that this too is an autosomal bivalent, with the smaller part, linked by the darker staining cytoplasm, being the X chromosome. Thus the sex chromosome element in this species is a trivalent with the X chromosome binding to an autosomal bivalent by means of part of the cytoplasm.

A. bonvouloiri Harold, 1860

Published information: none. 2n = 18 + Xy (♂), XX (♀). Male mitotic chromosomes, from mid-gut, are shown in Fig. 1, g (plain) and h (C-banded, a different nucleus from the same beetle). Karyotypes from these nuclei are shown in Fig. 2, f, g and a further C-banded karyotype, from a nucleus more heavily treated, is shown in Fig. 2, h. The two longest pairs of autosomes are submetacentric, with the short arms with secondary constrictions and darkly staining following moderate C-banding (Fig. 2, g), but much less so after stronger treatment (Fig. 2, h). Pair 3 is a fairly long metacentric with a strong centromeric C-band while pair 4 is a smaller metacentric with the C-banding extending along its short arm

Fig. 2, a-h. Mitotic chromosomes from mid-gut cells of *Aphodius abdominalis* (**a-e**) and *A. bonvouloiri* (f-h), arranged as karyotypes. **a** - ♂, plain. **b** - the same nucleus, C-banded (nuclei shown in Fig. 1, a, b). **c** - ♂, plain. **d** - ♀, plain. **e** - the same nucleus, C-banded (nuclei shown in Fig. 1, c, d). **f** - *A. bonvouloiri*, ♂, plain (nucleus shown in Fig. 1, g). **g** - *A. bonvouloiri*, ♂, lightly C-banded (nucleus shown in Fig. 1, h). **h** - *A. bonvouloiri*, ♂, heavily C-banded. Bar = 5μm.

following moderate treatment. Pairs 5-9 are acrocentrics steadily decreasing in size, with discrete centromeric C-bands. The X chromosome is subacrocentric, about the same length as autosome 5, and with a heavy centromeric C-band. The y chromosome is submetacentric, smaller than autosome 9. No preparations of meiosis are available.

Discussion

Comparison of the autosomes of *A. abdominalis* and *A. bonvouloiri* reveals a series of differences and similarities very normal when related species are considered. Thus the sequence of chromosome lengths along the karyotypes is very similar, though the decrease between pairs 3 and 4 appears more marked in *A. bonvouloiri*. There is no hint of NORs on autosome 1 and 2 of *A. abdominalis*, but there may be a NOR on autosome 4 of both species. *A. abdominalis* appears to have a distinct NOR on autosome 5, lacking in *A. bon-*

vouloiri. These results suggest that there are a number of translocational differences between these karyotypes.

The striking difference between these species is the lack of any y chromosome in *A. abdominalis*. In this feature it is clearly different not only from *A. bonvouloiri*, the only other species known in subgenus *Agolius*, but from all other *Aphodius* species whose karyotypes are known as well, unless the absence of a y chromosome in *A. nemoralis* is genuine. The association of the X chromosome with an autosomal bivalent at first division of meiosis is certainly very unusual, and may even be unique as far as is known. X0 sex chromosome systems are frequent in the coleopteran suborder Adephaga, where the X chromosome normally stands alone at first division of meiosis (e.g. *Stictotarsus griseostriatus* (De Geer, 1774) (Dytiscidae) (Angus, 2008)) but relatively unusual in the Polyphaga. Examples of X0 sex chromosomes in the Polyphaga include the Hydraenidae where Angus and Diaz Pazos (1991) illustrate unpaired X chromosomes at first metaphase of meiosis in species of *Hydraena* Kugelann, 1794 *Ochthebius* Leach, 1815 and *Limnebius* Leach, 1815 and Cantharidae where James and Angus (2006) illustrate unpaired X chromosomes at first metaphase of meiosis in larvae of *Cantharis rufa* Linnaeus, 1758. James and Angus demonstrated that the segregation of the X chromosome in meiosis of *C. rufa* was prereductional, i.e. the chromosome moves to one pole at first anaphase, and this was shown to be the case in the cantharoid family Lycidae by Virkki (1978), who suggested that this was retained from an ancestral Xy_p sex chromosome system. However, de Gambardella and de Vaio (1978) described an X0 system with postreductional segregation of the X chromosome in the Uruguayan cantharid *Chauliognathus scriptus* (Germar, 1824). Postreductional segregation of the X chromosome involves the X chromosome dividing mitotically at first metaphase of meiosis and the single-stranded daughter X chromosome then going to one pole at the end of the second division. The association of the X chromosome of *A. abdominalis* with an autosomal bivalent raises the question as to whether this species might exhibit postreductional segregation of the X, with one single-stranded daughter going to each pole at first meiotic division, accompanied by one autosome from the associated bivalent. This would repay further investigation.

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